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ARMSTRONG LABORATORY

EVALUATION OF THE TOXIC EFFECTS OF A 90-DAY CONTINUOUS EXPOSURE OF RATS TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Lt Col, USAF, BSC
Deputy Director, Toxicology Division
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A water-in-oil emulsion is being used in submarines as a hydraulic fluid under high pressure conditions. Acute toxicity tests using this hydraulic fluid indicated minimal toxicity. A continuous 90 day inhalation study was initiated to determine the possible toxic hazard that might be encountered from continuous exposure to Naval personnel. The test animals were exposed to either 0.2 or 1.0 mg hydraulic fluid/m^3. No treatment related effects were noted in body weights, organ weights, or clinical chemistry parameters at the conclusion of the study. Histopathology examination of the test animals revealed no lesions related to exposure. Under conditions of this study, no undue health hazard would be expected from exposures of 1.0 mg hydraulic fluid/m^3 or less.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc. This document serves as a final report on the toxic effect of continuous exposure to water-in-oil hydraulic fluid emulsion. The research described in this report began in June 1990 and was completed in May 1991 under U.S. Air Force Contract Nos. F33615-85-C-0532 and F33615-90-C-0532 (Study Nos. USN 0-81 2 and N08). Maj. James N. McDougal served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

This study was sponsored by the U.S. Navy under the direction of CAPT David A. Macys, MSC, USN. The work was supported by the Naval Medical Research and Development Command Task M0096 004 0006. Opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

APS	Aerodynamic particle sizer
C	Celsius
cm	Centimeter
dyn	Dyne
F-344	Fischer 344 (rats)
g	Gram
h	Hour
IR	Infrared
kg	Kilogram
L	Liter
m	Meter
m^2	Meter squared
m^3	Meter cubed
mg	Milligram
MMAD	Mass median aerodynamic diameter
µm	Micrometer
N	Number
NMRI/TD	Naval Medical Research Institute, Toxicology Detachment
p	Probability
psi	Pounds per square inch
S	Newtons seconds/m^2
sec	Second
SEM	Standard error of the mean

SECTION 1

INTRODUCTION

The Navy is interested in a commercial water-in-oil emulsion for use in submarine high-pressure, internal hydraulic fluid systems. The water-in-oil emulsion class of compounds consists of stable emulsions that contain 40% water homogeneously dispersed as micronic or submicronic size droplets in a 60% continuous oil phase. The oil phase is formulated from a high quality petroleum-based oil compound with special additives to give the final product added lubricity, corrosion protection, emulsion stability, and resistance to bacterial and fungal contamination. Chemical analyses of four water-in-oil hydraulic fluids previously studied in this laboratory indicated that ethylene glycol concentrations were approximately two percent (Kinkead et al., 1987).

A 90-day continuous exposure to a hydraulic fluid containing approximately 35% ethylene glycol resulted in increased relative liver weights at both test concentrations (10 and 100 mg/m^3) in female rats only (Wall et al., 1990). At the high concentration, increased numbers of pulmonary macrophages were noted in rats, rabbits, and hamsters. It has been reported (Roberts and Seibold, 1969) that macaque monkeys receiving ethylene glycol in drinking water (0.25 to 10%) developed renal pathologic effects resulting mainly from deposition of calcium oxalate crystals. However, kidney lesions were not noted in the above inhalation study

Previously conducted limit tests (oral, dermal, and inhalation) using water-in-oil hydraulic fluid emulsions indicated minimal toxicity (Kinkead et al , 1987) However, the inhalation exposures were low airborne concentrations due to the difficulty of aerosolizing these emulsions using conventional aerosol generators. The development of an aerosol generator (Kimmel and Leahy, 1990) capable of operating at high pressure (1000 psi) allowed for inhalation exposure evaluations at higher aerosol concentrations As these emulsions were scheduled to undergo sea trials in mid-1990, it was important to evaluate the possible toxic hazard that might be encountered during long-term continuous exposure to Naval personnel Aerosol inhalation exposures are hazards aboard Naval vessels where the crew's working and living environment are often the same.

The Naval Medical Research Institute, Toxicology Detachment (NMRI/TD) has provided information on industrial hygiene surveys aboard ships that indicated operationally observed aerosol concentrations of 0.2 mg/m^3 maximum (0.1 mg/m^3 average) for glycol-based hydraulic fluids Based on these industrial hygiene data, a continuous exposure to 0.2 mg/m^3 was chosen as the low-concentration exposure and 1.0 mg/m^3 as the high-concentration exposure. The low

concentration simulates realistic shipboard conditions, whereas the high concentration might be expected to simulate worst-case conditions.

This study was designed to determine and evaluate the potential toxic effects from a 90-day continuous exposure of water-in-oil hydraulic fluid aerosol to rats in Thomas Dome inhalation chambers. An air-exposed control group was also maintained under identical conditions.

SECTION 2

MATERIALS AND METHODS

ANIMALS

Upon receipt from Charles River Breeding Labs (Raleigh, NC), male and female Fischer 344 (F-344) rats were quality control tested prior to use in the studies. All rats were identified by tail tattoo. The rats were randomized using a proprietary modular software system (PATH/TOX® System, Xybion Medical Systems, Cedar Knoll, NJ) which assigned animals to groups. They were group housed (three per cage) in clear plastic cages with wood-chip bedding prior to the study. The rats (11 weeks of age at initial exposure) were then individually housed in wire-mesh stainless steel cages and assigned to specific exposure cage locations. Automatic water and feed (Purina Formulab #5008) were available ad libitum except when food was removed for 12 h prior to sacrifice. Ambient temperatures were maintained at 21 to 25 °C and the light/dark cycle was set at 12-h intervals (light cycle starting at 0700 h).

TEST MATERIAL

The water-in-oil hydraulic fluid emulsion was supplied by NMRI/TD. It was produced by E.F. Houghton and Company under the trade name Houghto-Safe® 5047F. The emulsion is described in the Material Safety Data Sheet as being 30 to 60% mineral oil (CAS No. 64741-89-5), 1 to 10% ethylene glycol (CAS No. 107-21-1), and the remainder water. Figure 1 is a typical infrared (IR) spectrum of the supplied hydraulic fluid emulsion. The primary use of IR spectra was for qualitative analysis of the supplied test material before use and for comparison with the spectra of pure mineral oil, ethylene glycol, and exposure aerosol droplets to determine the composition of these droplets. Pertinent physical properties of the test material are as follows:

Boiling point (°C)	101.70
Specific Gravity ($H_2O = 1$)	0.92
% volatiles by volume	30 to 60
pH	9.00
Absolute viscosity	8.3×10^{-2} (N x S)/m^2
Surface tension	35 dyn/cm
Appearance	white, milky fluid

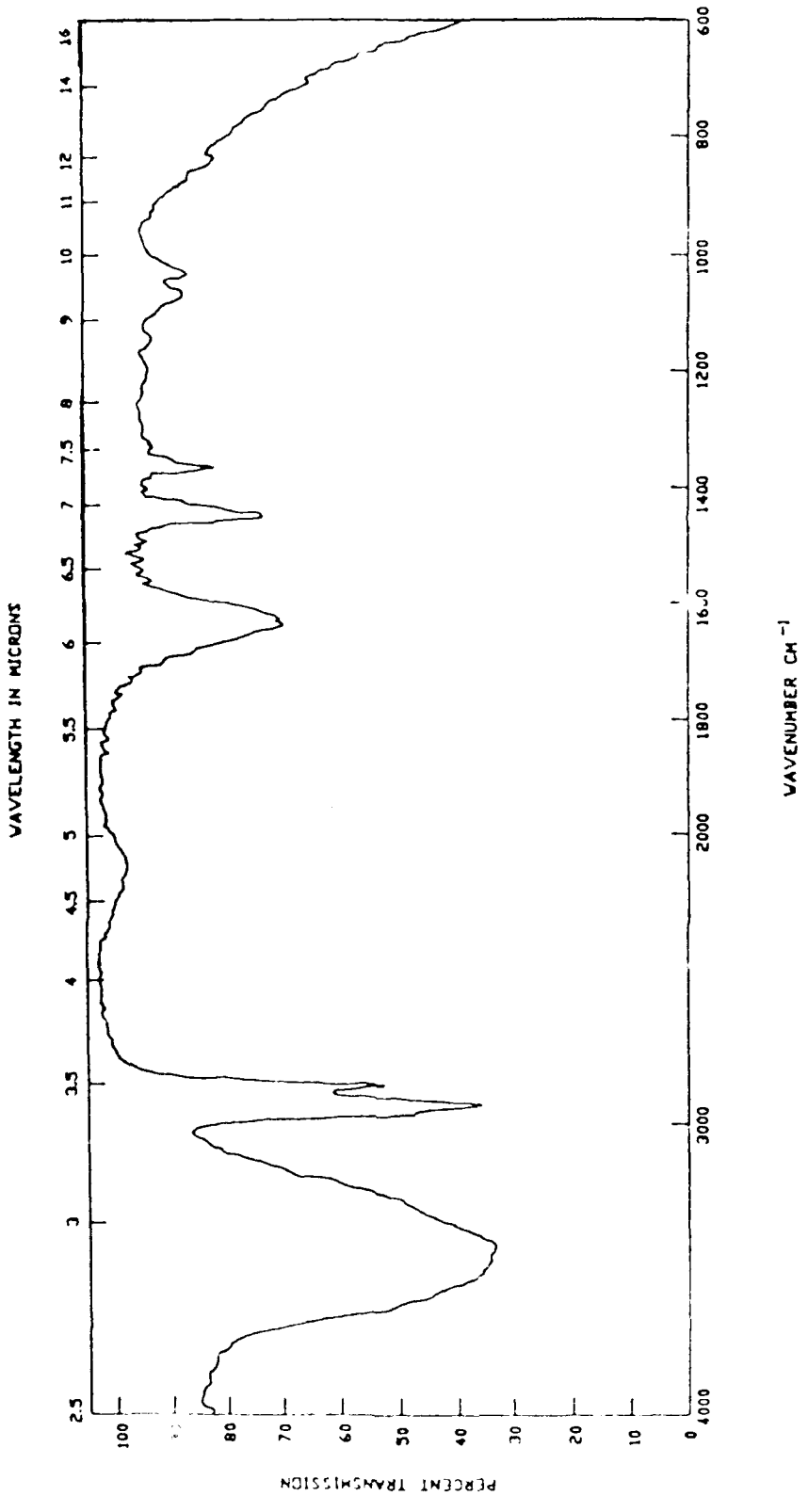

Figure 1. Infrared Spectrum of Houghto-Safe® 5047F Generated Using a Beckman Acculab 4 Infrared Spectrophotometer.

EXPOSURE REGIMEN AND RESPONSE ASSESSMENT

Groups of 60 male and 60 female F-344 rats were placed in 25-m^3 Thomas Dome chambers (Thomas, 1965) and exposed continuously (23 h/day) to either air alone, or 0.2 or 1.0 mg water-in-oil hydraulic fluid emulsion/m^3 for 90 days. Records were maintained for body weights, signs of toxicity, and mortality. Ten rats per sex per group (including controls) were sacrificed following 30, 60, and 90 days of exposure. Additional groups of 10 rats per sex per group were sacrificed at 30, 60, and 90 days postexposure. The high concentration and control animals sacrificed following the termination of the inhalation study had a complete histopathologic examination. Because no treatment-related lesions were found in the high concentration animals, histological examinations were not performed on tissues from the low concentration group. The rats serially sacrificed during exposure or postexposure had only lungs and kidneys examined histopathologically. Tissues for histopathologic examination were fixed in 10% neutral-buffered formalin, trimmed, and further processed via routine methods for hematoxylin and eosin-stained paraffin-embedded sections (Luna, 1968).

Wet tissue weights were determined on adrenals, brain, heart, kidney, liver, lungs, ovaries/testes, spleen, and thymus on all animals sacrificed at exposure termination. Additionally, blood was drawn for hematology and clinical chemistry assays. Erythrocytes were enumerated on a Coulter counter (Coulter Electrics, Hialeah, FL), and sera for clinical chemistry evaluation were assayed on an Ektachem 700XR (Eastman Kodak, Rochester, NY). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures.

TEST MATERIAL GENERATION AND ANALYSIS

The test material was generated under conditions simulating a pin-point breach in a hydraulic system. Aerosol of the hydraulic fluid emulsion was generated through a micro-orifice misting nozzle-hydraulic pump system in which the test material was maintained at 1000 psi pressure (Kimmel and Leahy, 1990). Separate generators were used for each concentration tested.

Continuous monitoring of aerosol concentration was performed by near-forward angle light-scattering (RAM-S, MIE, inc., Bedford, MA). The daily concentration was calculated from the RAM-S continuous analysis as processed by the Toxic Hazards Research Unit data acquisition system from 1300 to 1400 individual data points. The nonexposure period needed for animal maintenance was not included in the calculations, therefore, the daily average represents only the approximately 23 h exposure period. Aerodynamic particle size was determined twice daily using an aerodynamic particle sizer (APS 33B, TSI Inc., St. Paul, MN). Particle size was confirmed weekly using a Cascade Impactor. A Miran IA (Foxboro, S. Norwalk, CN) longpath IR was used for continuous ethylene glycol analysis. A detailed description of the sampling systems and calibration procedures is described in Kimmel and Leahy, 1990.

STATISTICAL ANALYSIS

Comparisons of mean body weights were performed using a two-factorial analysis of variance A two-factorial analysis of variance with multivariate comparisons was used to analyze clinical chemistry and organ weight data (Barcikowski, 1983; Dixon, 1990) The histopathology data were analyzed using Yates' corrected Chi-square (Zar, 1974) Chamber concentrations were analyzed by standard statistical methods using BMDP 1-D (Dixon, 1990)

SECTION 3

RESULTS

The ethylene glycol vapor concentration present in the 0 2 mg hydraulic fluid/m^3 atmosphere was below detectable limits Ethylene glycol concentration in the high exposure atmosphere was only slightly above minimal detectable limits The average weekly mean concentration of ethylene glycol in this exposure chamber ranged from 0 2 to <0 4 mg/m^3 for an overall average concentration of <0 3 mg/m^3

A comparison of the IR spectra of freshly collected aerosol droplets with those of pure mineral oil, ethylene glycol, and neat 5047F hydraulic fluid indicated that the aerosol droplets were virtually pure mineral oil, the spectra being void of absorbance bands attributable to water or ethylene glycol Details of the atmosphere analysis are described in Kimmel and Leahy (1990)

Generation of desired aerosol concentrations occurred with few problems, and mean chamber concentrations were maintained within 10% of the target concentrations Mean concentrations for each exposure chamber, including high and low daily mean concentrations, are provided in **Table 1**. There was good agreement in comparison of the mass median aerodynamic diameter (MMAD) values obtained by the two sizing methods, however, the values in the table are those obtained using the aerodynamic particle sizer (APS)

TABLE 1. STATISTICAL ANALYSIS OF WATER-IN-OIL HYDRAULIC FLUID EMULSION CONCENTRATIONS INHALED BY MALE AND FEMALE F-344 RATS FOR 90 DAYS

Target Concentration, mg/m^3	0 20	1 00
Mean Concentration, mg/m^3[a]	0 20	1 04
Standard Error	< 0 01	< 0 01
Lowest Daily Mean, mg/m^3	0 18	0 95
Highest Daily Mean, mg/m^3	0 26	1 13
Aerosol Size Distributions[b]		
Mean MMAD (µm)[a]	2 80	3 09
Geometric standard deviation (µm)	1 42	1 45

[a]Mean of daily means
[b]APS data

Mean body weights of the hydraulic fluid exposed rats were not statistically different from the control animals at any of the weighing periods (Figure 2 and Appendix A) Clinical chemistry values for male and female rats evaluated following termination of the 90-day exposure period are listed in **Tables 2** and **3**, respectively No treatment-related effects were noted in any parameters examined

TABLE 2. MEAN[a] SERUM CHEMISTRY VALUES OF MALE F-344 RATS FOLLOWING A 90-DAY CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

	Control			0.2 mg/m³			1.0 mg/m³		
Glucose	167.2	±	6.0	175.2	±	4.0	165.3	±	5.5
BUN	13.1	±	0.6	12.8	±	0.6	13.9	±	0.7
Creatinine	0.3	±	<0.1	0.3	±	<0.1	0.3	±	<0.1
Sodium	143.0	±	0.4	143.5	±	0.3	142.5	±	0.4
Potassium	5.0	±	0.2	4.9	±	0.1	5.2	±	0.1
Chloride	97.8	±	0.3	98.3	±	0.4	98.3	±	0.3
Calcium	11.4	±	0.1	11.5	±	0.1	11.4	±	0.1
Phosphorus	8.3	±	0.2	8.3	±	0.2	8.0	±	0.3
Total Protein	6.2	±	<0.1	6.3	±	<0.1	6.2	±	<0.1
Albumin	3.3	±	<0.1	3.3	±	<0.1	3.4	±	<0.1
AST	73.3	±	5.3	69.5	±	3.2	78.5	±	3.9
ALT	51.9	±	2.1	52.9	±	2.5	59.1	±	3.3
Alkaline Phosphatase	163.8	±	13.8	152.7	±	12.8	175.0	±	14.5

[a]Mean ± SEM, N = 10

TABLE 3. MEAN[a] SERUM CHEMISTRY VALUES OF FEMALE F-344 RATS FOLLOWING A 90-DAY CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

	Control			0.2 mg/m³			1.0 mg/m³		
Glucose	134.4	±	4.3	136.1	±	4.5	135.0	±	3.3
BUN	16.0	±	0.6	14.8	±	0.6	15.7	±	0.5
Creatinine	0.4	±	<0.1	0.4	±	<0.1	0.4	±	<0.1
Sodium	143.6	±	0.4	143.5	±	0.3	143.2	±	0.2
Potassium	5.5	±	0.2	5.3	±	0.1	5.4	±	0.1
Chloride	100.7	±	0.4	100.1	±	0.4	100.8	±	0.5
Calcium	11.2	±	0.1	11.5	±	0.1	11.3	±	0.1
Phosphorus	8.0	±	0.3	7.5	±	0.4	7.6	±	0.3
Total Protein	6.4	±	0.1	6.6	±	0.1	6.5	±	0.1
Albumin	3.4	±	0.1	3.5	±	<0.1	3.4	±	0.1
AST	76.0	±	3.9	76.4	±	3.4	73.0	±	1.6
ALT	50.9	±	2.5	56.2	±	2.3	48.1	±	1.5
Alkaline Phosphatase	101.5	±	6.2	123.7	±	16.9	122.7	±	6.5

[a]Mean ± SEM, N = 10

Organ weights examined at the conclusion of the continuous inhalation exposure revealed no treatment-related differences in treated animals when compared with their respective control groups (**Tables 4** and **5**).

TABLE 4. ORGAN WEIGHTS (g)[a] AND ORGAN-TO-BODY WEIGHT RATIOS (%) OF MALE F-344 RATS FOLLOWING A 90-DAY CONTINUOUS INHALATION EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

	Control	0.2 mg m³	1.0 mg/m³
Kidney	2 16 ± 0 05	2 16 ± 0 06	2 21 ± 0 05
Ratio[b]	0 61 ± 0 01	0 61 ± 0 01	0 63 ± 0 01
Heart	0 97 ± 0 02	0 99 ± 0 02	1 03 ± 0 03
Ratio	0 28 ± <0 01	0 28 ± <0 01	0 30 ± 0 01
Brain	1 85 ± 0 03	1 87 ± 0 03	1 89 ± 0 02
Ratio	0 53 ± 0 01	0 53 ± 0 01	0 54 ± 0 01
Liver	10 32 ± 0 33	10 45 ± 0 30	10 62 ± 0 31
Ratio	2 93 ± 0 06	2 97 ± 0 05	3 04 ± 0 06
Spleen	0 62 ± 0 02	0 60 ± 0 01	0 63 ± 0 01
Ratio	0 18 ± <0 01	0 17 ± <0 01	0 18 ± <0 01
Thymus	0 27 ± 0 02	0 26 ± 0 01	0 30 ± 0 02
Ratio	0 08 ± 0 01	0 07 ± <0 01	0 09 ± <0 01
Lungs	1 62 ± 0 05	1 62 ± 0 04	1 74 ± 0 04
Ratio	0 46 ± 0 02	0 46 ± 0 01	0 50 ± 0 01
Adrenals	0 05 ± <0 01	0 05 ± <0 01	0 05 ± <0 01
Ratio	0 01 ± <0 01	0 01 ± <0 01	0 01 ± 0 01
Testes	3 04 ± 0 04	2 92 ± 0 07	2 94 ± 0 05
Ratio	0 87 ± 0 02	0 83 ± 0 02	0 84 ± 0 01
Whole Body[c]	352 4 ± 7 4	351 4 ± 7.2	348 8 ± 5 3

[a] Mean ± SEM N = 10
[b] Organ weight/body weight × 100
[c] Fasted weights

TABLE 5. ORGAN WEIGHTS (g)[a] AND ORGAN-TO-BODY WEIGHT RATIOS (%) OF FEMALE F-344 RATS FOLLOWING A 90-DAY CONTINUOUS INHALATION EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

	Control	0.2 mg m^3	1.0 mg m^3
Kidney	1 23 ± 0 03	1 21 ± 0 02	1 17 ± 0 02
Ratio[b]	0 68 ± 0 02	0 67 ± 0 01	0 65 ± 0 01[c]
Heart	0 65 ± 0 02	0 65 ± 0 01	1 60 ± 0 01
Ratio	0.36 ± 0 01	0 36 ± 0 01	0 33 ± 0 01[c]
Brain	1 75 ± 0 01	1 75 ± 0 01	1.75 ± 0 02
Ratio	0 97 ± 0 02	0 96 ± 0 01	0 97 ± 0 01[c]
Liver	4 50 ± 0 12	4 74 ± 0 07	4 52 ± 0 09
Ratio	2 49 ± - 0 04	2 61 ± 0 05	2 48 ± 0 04[c]
Spleen	0 41 ± 0 01	0 40 ± 0 01	0 41 ± 0 01
Ratio	0 23 ± <0 01	0 22 ± <0 01	0 23 ± <0 01[c]
Thymus	0 22 ± 0 01	0 20 ± 0 01	0 22 ± 0 01
Ratio	0.12 ± 0 01	0 11 ± 0 01	0 12 ± <0 01[c]
Lungs	1 13 ± 0 03	1.15 ± 0 03	1 10 ± 0 04
Ratio	0 63 ± 0 01	0 63 ± 0 02	0 60 ± 0 02[c]
Adrenals	0 05 ± <0 01	0 05 ± <0 01	0 05 ± <0 01
Ratio	0 03 ± <0 01	0 03 ± <0 01	0 03 ± <0 01[c]
Ovaries	0 08 ± <0 01	0 08 ± <0 01	0 08 ± <0 01
Ratio	0 04 ± <0 01	0 05 ± <0 01	0 05 ± <0 01[c]
Whole Body[c]	180 8 ± 3 6	181 5 ± 1 7	181 0 ± 2.5[c]

[a]Mean ± SEM, N = 10
[b]Organ weight/body weight × 100
N = 9
[c]Fasted weights

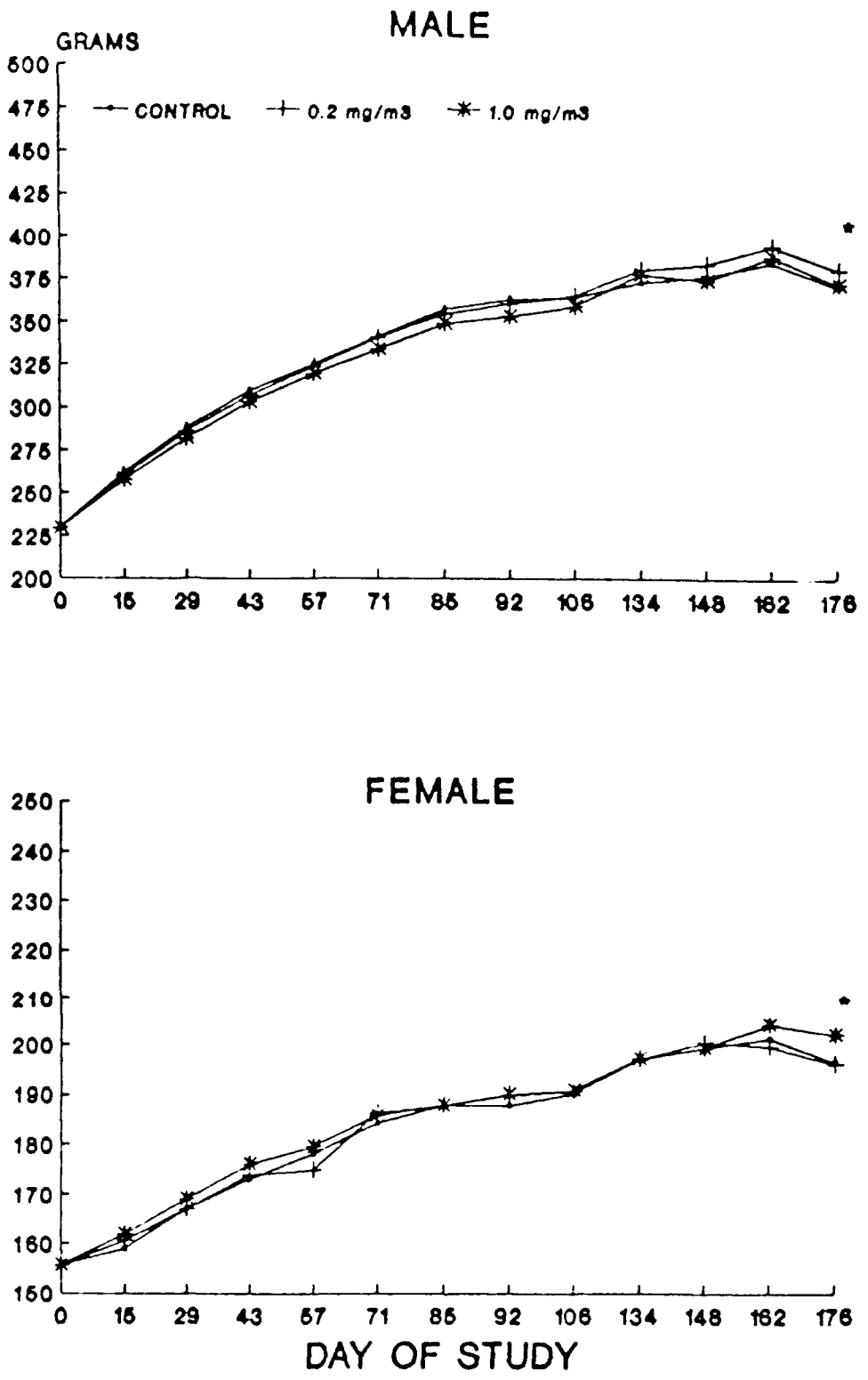

Figure 2. Effect of a 90-Day Continuous Inhalation Exposure to Water-In-oil Hydraulic Fluid Emulsion on Mean Body Weights of Male and Female F-344 Rats.

At necropsy, all rats were in good general condition and grossly observed lesions were rare and not treatment-related. A male rat sacrificed immediately following 90 days exposure to 1.0 mg/m^3 had a 0.5 cm diameter mass associated with the left external ear canal. A female rat sacrificed immediately following 90 days exposure to 1.0 mg/m^3 had a 0.5 cm raised yellow focus on the liver.

Histological examination determined that the ear lesion was a Zymbal gland tumor composed of squamous and sebaceous components and the liver lesion was a herniation of the diaphramatic lobe.

Microscopic findings of kidneys and lungs taken at serial sacrifice and tissues examined immediately following the 90-day continuous inhalation exposure are listed in **Tables 6** through **11**. Incidence data indicate the frequent occurrence of renal tubular alteration (defined as degenerative and regenerative changes) and periglomerular sclerosis as an age-related change affecting the kidneys of predominantly male rats and pulmonary vascular mineralization involving both sexes of the control and high-concentration groups. Renal laminar concretions were noted in at least 90% of all animals examined throughout the study. An increase in interstitial inflammation was noted at 30-days postexposure in female rats only. Pertinent lesions in tissues examined immediately following the 90-day inhalation exposure included myocardial degeneration and vascular mineralization observed in both sexes and portal inflammation in the liver, primarily in the female rats (**Table 8**). Statistical analysis did not disclose differences in the occurrence of histopathologic lesions between high-concentration and control animals. Because no lesions attributed to the test material exposure were found in the high-concentration group, tissues from the low-concentration exposure group were not examined histologically.

TABLE 6. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGIC FINDINGS IN MALE AND FEMALE F-344 RATS FOLLOWING 30-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ/Lesion	Control	1.0 mg m^3	Control	1.0 mg/m^3
Kidneys:				
Laminar concretions	100	100	100	100
Fibrosis	20	0	0	0
Interstitial inflammation	20	10	10	0
Tubular alteration	90	100	0	0
Periglomerular sclerosis	20	30	0	0
Lungs:				
Vascular mineralization	20	20	50	70

TABLE 7. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGIC FINDINGS IN MALE AND FEMALE F-344 RATS FOLLOWING 60-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ Lesion	Control	1.0 mg/m³	Control	1.0 mg/m³
Kidneys:				
Laminar concretions	100	100	100	100
Fibrosis	0	10	0	0
Interstitial inflammation	30	30	0	0
Tubular alteration	100	80	0	0
Periglomerular sclerosis	50	40	0	0
Lungs:				
Vascular mineralization	50	40	30	50

TABLE 8. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGIC FINDINGS IN MALE AND FEMALE F-344 RATS FOLLOWING 90-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ/Lesion	Control	1.0 mg/m³	Control	1.0 mg/m³
Liver:				
Portal inflammation	0	20	60	50
Kidneys:				
Laminar concretions	100	100	100	90
Interstitial inflammation	40	50	10	0
Tubular alteration	90	100	0	10
Periglomerular sclerosis	100	100	10	0
Lungs:				
Vascular mineralization	90	70	40	60
Heart:				
Vascular mineralization	10	20	20	0
Myocardial degeneration	80	70	70	50

TABLE 9. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGY 30 DAYS POSTEXPOSURE FOLLOWING 90-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ/Lesion	Control	1.0 mg/m^3	Control	1.0 mg/m^3
Kidneys:				
Laminar concretions	100	100	100	100
Interstitial inflammation	60	80	0	50[a]
Tubular alteration	100	100	10	10
Periglomerular sclerosis	90	90	40	30
Lungs:				
Vascular mineralization	100	70	70	60

[a] = Significantly different from control, < 0.01

TABLE 10. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGY 60 DAYS POSTEXPOSURE FOLLOWING 90-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ/Lesion	Control	1.0 mg/m^3	Control	1.0 mg/m^3
Kidneys:				
Laminar concretions	100	100	90	100
Interstitial inflammation	100	80	10	10
Tubular alteration	90	100	40	40
Periglomerular sclerosis	100	90	30	50
Lungs:				
Vascular mineralization	80	100	80	100

TABLE 11. INCIDENCE (%) OF KIDNEY AND LUNG HISTOPATHOLOGY 90 DAYS POSTEXPOSURE FOLLOWING 90-DAYS CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION (N = 10)

	Male		Female	
Organ/Lesion	Control	1.0 mg/m^3	Control	1.0 mg m^3
Kidneys:				
Laminar concretions	100	100	100	100
Fibrosis	20	30	0	0
Interstitial inflammation	100	80	10	0
Tubular alteration	100	100	20	0
Periglomerular sclerosis	90	100	30	40
Lungs:				
Vascular mineralization	80	80	60	90
Interstitial fibrosis	0	10	0	0

SECTION 4

DISCUSSION

This study supports the conclusions derived from acute studies indicating that water-in-oil hydraulic fluid emulsions are associated with minimal toxicity (Kinkead et al , 1987) The emulsion used in this study (0 to 10% ethylene glycol) did not produce the toxic effects observed in the previous study using a hydraulic fluid containing 35% ethylene glycol (Wall et al , 1990) Ethylene glycol vapor concentrations, detectable only in the high concentration atmosphere, were several orders of magnitude below the threshold limit value of 127 mg/m^3 recommended by the American Conference of Governmental Industrial Hygienists (1990)

No treatment-related changes were noted in mean body weights, organ weights, or clinical chemistry parameters The lack of significant differences in occurrence and severity of histopathologic lesions between the test and control groups suggested that these findings represent normal background or aging changes The most significant difference between male and female groups was the incidence of many renal lesions

The glomerular, tubular, and inflammatory changes can be attributed to chronic progressive nephrosis of F-344 rats This condition affects primarily male rats beginning at an early age and progresses to severe lesions by 18 to 30 months of age Hyaline droplets are frequently observed with this condition and although not listed as a lesion in the tables, they were observed in the renal tubular epithelium of all male rats and were absent in females

The renal changes which occurred at a high incidence in both controls and test chemical exposed animals are common background lesions in F-344 rats Lamellated concretions (microliths) are particularly common among female rats, and may be observed as early as seven weeks of age (Montgomery and Seely, 1990) Hyaline droplet accumulation in renal tubules occurs primarily in male rats A wide variety of hydrocarbon-containing chemicals has the ability to exacerbate hyaline droplet accumulation and cause more extensive degeneration of the proximal tubule epithelium (Alden, 1986) The background lesions are distinguished from lesions that might be expected to occur as a result of exposure to a material containing ethylene glycol in that calcium oxalate crystals, tubular epithelial necrosis, and vascular congestion were not present

The only neoplasm observed was a Zymbal gland tumor in one male rat These occur infrequently as spontaneous neoplasms and can be induced by carcinogens This neoplasm was interpreted as spontaneous in this case. Similarly, the herniation of the diaphramatic liver lobe in one female rat was interpreted as a spontaneous change

Under the conditions of this study, the water-in-oil hydraulic fluid emulsion would not be considered to be toxic. These findings suggest that the 1 mg water-in-oil hydraulic fluid emulsion/m^3 concentration would present no toxic hazard upon prolonged exposure.

SECTION 5

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APPENDIX A

MEAN[a] BODY WEIGHTS (g) OF F-344 RATS FROM A 90-DAY CONTINUOUS EXPOSURE TO WATER-IN-OIL HYDRAULIC FLUID EMULSION

Day	Control	0.2 mg/m³	1.0 mg/m³
	Male		
0	229.8 ± 1.0 (60)	229.8 ± 1.0 (60)	229.8 ± 1.0 (60)
14	261.9 ± 1.6 (60)	260.2 ± 1.5 (60)	257.5 ± 1.5 (60)
28	287.9 ± 1.8 (60)	286.2 ± 1.8 (60)	282.2 ± 1.7 (60)
42	309.8 ± 2.3 (50)	306.8 ± 1.9 (50)	303.1 ± 2.2 (50)
56	325.1 ± 2.5 (50)	323.7 ± 2.2 (50)	319.6 ± 2.5 (50)
70	341.7 ± 3.2 (40)	340.8 ± 3.0 (40)	334.1 ± 3.0 (40)
84	357.3 ± 3.3 (40)	354.4 ± 3.1 (40)	348.9 ± 3.1 (40)
91	362.8 ± 3.3 (40)	360.7 ± 3.1 (40)	353.3 ± 3.0 (40)
105	364.4 ± 3.8 (30)	365.6 ± 3.6 (30)	359.3 ± 3.3 (30)
119	368.7 ± 4.6 (20)	370.7 ± 3.9 (20)	366.9 ± 4.6 (20)
133	373.1 ± 5.1 (20)	380.8 ± 3.7 (20)	378.1 ± 5.0 (20)
147	377.1 ± 5.0 (20)	383.9 ± 4.0 (20)	374.6 ± 6.6 (20)
161	384.9 ± 6.1 (10)	394.8 ± 5.5 (10)	388.8 ± 7.1 (10)
	Female		
0	155.8 ± 0.6 (60)	155.8 ± 0.6 (60)	155.8 ± 0.6 (60)
14	159.0 ± 1.0 (60)	160.5 ± 0.8 (60)	161.9 ± 0.8 (60)
28	167.2 ± 1.0 (60)	167.1 ± 0.9 (60)	169.0 ± 0.9 (60)
42	173.0 ± 1.3 (50)	173.8 ± 1.1 (50)	175.9 ± 1.1 (50)
56	178.0 ± 1.3 (50)	174.9 ± 2.3 (50)	179.5 ± 1.2 (50)
70	184.1 ± 1.6 (40)	186.2 ± 1.5 (40)	185.8 ± 1.2 (40)
84	187.7 ± 1.7 (40)	187.7 ± 1.2 (40)	187.7 ± 1.3 (40)
91	187.7 ± 1.7 (40)	189.7 ± 1.3 (40)	190.0 ± 1.3 (40)
105	190.1 ± 1.8 (30)	190.6 ± 1.3 (30)	190.7 ± 1.4 (30)
119	191.3 ± 2.1 (20)	190.9 ± 1.9 (20)	188.9 ± 1.7 (20)
133	197.0 ± 1.8 (20)	197.1 ± 1.8 (20)	197.1 ± 1.6 (20)
147	199.3 ± 1.8 (20)	200.3 ± 2.0 (20)	199.2 ± 1.8 (20)
161	201.2 ± 2.1 (10)	199.6 ± 1.9 (10)	204.0 ± 2.8 (10)

[a]Mean ± SEM (N)

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QUALITY ASSURANCE

The study, Evaluation of the Toxic Effects of a 90-Day Continuous Exposure of Rats to Water-in-Oil Hydraulic Fluid Emulsion, was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of these guidelines. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
July 23, 1990	Weigh, randomize study animals
July 24, 1990	Initiate 90-day exposure
August 13, 1990	Generation system: rotameter
August 14, 1990	Analytical system: grab filter
August 15, 1990	Analytical system: impactor
August 17, 1990	Analytical system: APS
August 21, 1990	Weigh study animals
August 22, 1990	30-day sacrifice
September 11, 1990	Analytical system: grab filter
September 12, 1990	Analytical system: impactor
September 13, 1990	Analytical system: hygrometer
September 14, 1990	Analytical system: APS
September 18, 1990	Weigh study animals
September 20, 1990	60-day sacrifice
October 9, 1990	Analytical system: grab filter
October 10, 1990	Analytical system: impactor
October 12, 1990	Analytical system: APS
October 15, 1990	Generation system: rotameter
October 24, 1990	90-day sacrifice
November 20, 1990	30-day post-exposure sacrifice
December 18, 1990	60-day post-exposure sacrifice
January 14, 1991	90-day post-exposure sacrifice
May 9-16, 1991	Final report audit

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M. G. Schneider
QA Coordinator
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Date 29 May 91